Effect of the chemical form of supranutritional selenium on selenium load and selenoprotein activities in virgin, pregnant, and lactating rats[1]

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ABSTRACT: Virgin, pregnant, and lactating rats were used to assess the influence of selenomethionine and selenocystine, fed at four to seven times the daily Se requirement (supranutritional), on Se load and selenoprotein activities. Female Sprague Dawley rats (n = 48; age = 13 wk), reared on a low-Se torula yeast diet, were assigned to one of three reproductive states (n = 16 per reproductive state) to occur simultaneously: virgin, pregnant, and lactating. Once reproductive state was achieved, rats were fed (ad libitum) either L-selenomethionine (n = 24) or L-selenocystine (n = 24) diets providing 2.0 μg Se/g of diet (as-fed basis) for 18 d, and then killed. Lactating rats consuming selenomethionine had the greatest Se concentration in the brain, with pregnant rats being intermediate, and virgin rats having the least ($P < 0.02$). When selenocystine was fed, the concentration of Se in the brain was greater ($P = 0.008$) in lactating rats, but not different ($P = 0.34$) between pregnant and virgin rats. Selenium concentrations in the heart, liver, lung, muscle, spleen, plasma, placenta, uterus, and fetus were greatest ($P < 0.001$) in rats consuming selenomethionine. Brain, kidney, and liver thioredoxin reductase, and brain, erythrocyte, kidney, and liver glutathione peroxidase activities did not differ ($P = 0.13$ to $P = 0.85$) between Se treatments. Lactating rats exhibited the greatest ($P < 0.006$) Se concentration in the heart, lung, muscle, plasma, and spleen compared with pregnant and virgin rats. Thioredoxin reductase was greatest ($P < 0.004$) in the brain of pregnant rats, greatest ($P < 0.004$) in the liver of lactating rats, and greater ($P < 0.03$) in the kidney of lactating and pregnant vs. virgin rats. Regardless of reproductive state, supranutritional Se (2.0 μg/g of diet) fed as selenocystine resulted in less Se load, and when fed as selenomethionine, was equally available for thioredoxin reductase synthesis as the Se in selenocystine. Independent of dietary Se chemical form, thioredoxin reductase activity was responsive to reproductive state.

Key Words: Reproduction, Selenium, Selenocystine, Selenomethionine, Supranutritional, Thioredoxin Reductase

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Introduction

Sodium-selenate and -selenite are the most common Se sources used to enhance dietary Se in livestock diets. Recently, Se-enriched yeast, an organic bound source, was approved for use in poultry (FDA, 2001), swine (FDA, 2003), and cattle (FDA, 2004) diets. The fortification of livestock diets with these manufactured Se sources is regulated and cannot exceed the equivalent of 0.3 μg of Se/g of feed (complete diet; FDA, 2004). Current restrictions do not curtail the use of naturally high-Se feedstuffs as a vehicle to deliver Se in excess of an animal's daily requirement, or as referred to hereafter, supranutritional. van Ryssen et al. (1989), Hintze et al. (2002), and Lawler et al. (2004) demonstrated the strategic use of supranutritional, organically bound Se (high-Se wheat grain) to rapidly enhance dietary Se, and subsequently, the overall Se load in livestock.

The Se chemical form in commonly fed high-Se feedstuffs (e.g., grains, legumes) has been suggested to be mainly selenomethionine, with some selenocystine/selenocysteine (Wu et al., 1997; Whanger, 2002). Dietary selenomethionine passes to the monogastric and ruminant duodenum relatively unchanged; however, due to ruminal microorganisms, dietary Se salts seem to be converted to predominantly selenocysteine (van Ryssen

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et al., 1989). Although well defined in male mammals, limited information is available defining the metabolic fate of selenomethioinine and seleno-cystine/-cysteine in pregnant and lactating females, especially when fed supranutritionally. We hypothesized that Se chemical form and female reproductive state would independently influence Se distribution and status in females consuming supranutritional Se for a brief period. To test this hypothesis, virgin, pregnant, and lactating rats were used to assess the effects of supranutritional L-selenomethionine and L-selenocystine on Se load and selenoprotein activities.

Materials and Methods

The Institutional Animal Care and Use Committee (Protocol No. SeWBBr1, USDA-ARS, Grand Forks, ND) reviewed and approved the use of the rats and their offspring as described herein. Female Sprague Dawley rats (n = 48) were obtained at 21 d of age (BW = 40 to 50 g; SASCO, Madison, WI), housed individually in suspended stainless-steel mesh cages (temperature = 22 ± 1.1°C; humidity = 50 ± 10%; light = 12 h), and reared on a torula yeast-based diet (ad libitum; Se = <0.02 μg/g), with no supplemental Se (28.8% torula yeast [Harlan Teklad, Madison, WI], 54.8% sucrose, 5.1% corn oil, 5.1% cellulose, 3.6% Se-deficient mineral mix [AIN 76], 1.2% calcium carbonate, 1.0% vitamin mix [AIN 76A], 0.3% DL-methionine, 0.1% choline bitartrate, 0.009% [500 IU/g] vitamin E acetate, 0.004% [0.1% mannitol] vitamin B_{12}, 0.0016% [500,000 IU/g] vitamin A palmitate, 0.001% menadione sodium bisulfate complex, and 0.00025% [400,000 IU/g] vitamin D3; DM basis). Throughout the rearing, breeding, and experimental periods, rats were provided deionized water ad libitum. At 13 wk of age, rats were assigned to one of three reproductive states to occur simultaneously: virgin (n = 16), pregnant (n = 16), or lactating (n = 16). Once the targeted reproductive state was achieved (described below), rats were fed (ad libitum) either a L-selenocystine (n = 24; Sigma-Aldrich, St. Louis, MO) or L-selenomethionine (n = 24; Sigma-Aldrich) Se chemical form treatment diet, providing 2.0 μg of Se/g of diet (as-fed basis; four to seven times the requirement for pregnant/lactating and virgin, respectively; NRC, 1995). Selenium forms were incorporated into the previously described torula yeast diet.

The simultaneous alignment of reproductive states is diagramed in Figure 1. Male rats were introduced into the female housing area 7 d before a scheduled breeding event to initiate cycling. For breeding, female rats were individually exposed to a male for 5 d, and day of conception was estimated based on identification of vaginal plug shed. Rats assigned to the lactating group were bred 21 d before commencement of Se form treatments. At the onset of parturition in the lactating group (moved to solid-bottom cages with bedding material), breeding was initiated in the pregnant group. Based on the order of parturition and conception for the lactating and pregnant groups, respectively, Se form treatment diets were assigned to each female rat in an alternating sequence. Simultaneously, treatments were randomly assigned to each rat in the virgin group (not exposed to males). Treatments were fed for 17 or 18 d depending on day of conception or parturition for the pregnant and lactating groups, respectively. For pregnant and lactating groups, conception and parturition occurred 1.4 (SD = 0.7) and 23.7 (SD = 0.5) d following d 1 of exposure to a male, respectively.

At trial termination, rats (including lactating group pups) were weighed, anesthetized (87 mg of ketamine/kg of BW and 13 mg of xylazine/kg of BW), and decapitated. The brain (whole), heart, kidneys, liver, a lung, muscle (tensor fasciae latae), uterus, and spleen were collected. Organ weights were measured for the heart, liver, and uterus, and the uterus was further dissected, emptied (all contents removed), and weighed. The fetuses and placentas from the pregnant rats and pups from the lactating rats were counted and individually weighed. Four pups were randomly selected from each litter, and livers were removed. All organs and tissues collected were wrapped in aluminum foil, snap frozen (liquid N), and stored (–60°C) until subsequent analysis.

Hydride generation atomic absorption spectrometry was used to analyze for Se (detection limit = 1 ng/mL of wet-ashed sample; intra- and interassay CV were <7 and <6%, respectively) following digestion of samples with nitric acid (Finley et al., 1996). All samples were analyzed in triplicate. A bovine liver standard (SRM 1577b; National Institutes of Standards and Technology, Gaithersburg, MD) was used to assess analysis accuracy. The method of Holmgren and Bjornstedt (1995), with the modifications of Hill et al. (1997), was used to estimate kidney, brain, and liver thioredoxin reductase activities following the reduction of 5,5-dithiobis [2-nitrobenzoic acid] giving two molecules of 5-thio-2-nitrobenzoic acid (absorbance = 412 nm) in the presence of NADPH. Thioredoxin reductase activity was determined by subtracting the time-dependent increase in absorbance at 412 nm in the presence of the aurothioglucose (20 μ*M*; thioredoxin reductase activity inhibitor) from total thioredoxin reductase activity. One unit of activity is described as 1 μmol of 5-thio-2-nitrobenzoic acid formed·min^{-1}·mg of $protein^{-1}$. The coupled enzyme method of Paglia and Valentine (1967) was used to measure kidney, brain, liver, and erythrocyte glutathione peroxidase activities using H_2O_2 as the substrate and NADPH as the source of reducing equivalents (absorbance = 340 nm). One unit of activity is described as 1 μmol of NADPH oxidized·min^{-1}·mg $protein^{-1}$ (Hintze et al., 2003). Protein was measured using the BioRad (Hercules, CA) protein assay kit (No. 500-0002; Coomassie brilliant blue G-250 binding assay; absorbance = 595). For enzyme activity and protein analyses, the intra- and interassay CV were <6 and <10%, respectively.

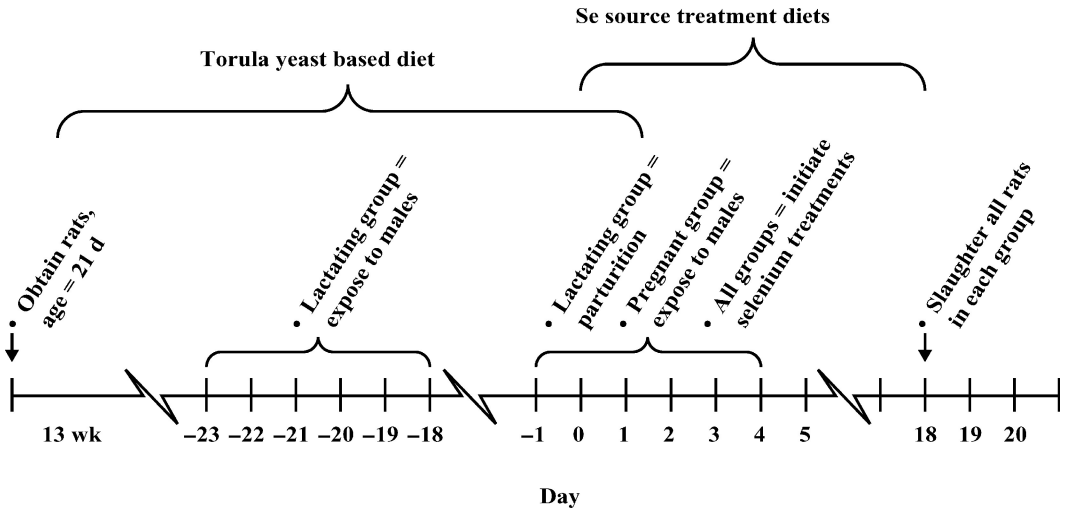

Figure 1. Timeline of breeding (exposure to males), Se chemical form treatment, and slaughter of female rats assigned to virgin, pregnant, and lactating groups.

Before statistical analysis, some individual rat data were eliminated due to lost sample identity (two virgin rats), loss of pregnancy (one rat), improper sample/tissue labeling (one lactating rat), or loss of pups (one rat). For analysis, the total individually treated rats in the selenomethionine group were seven virgin, seven pregnant, and seven lactating, and in the selenocystine group were seven virgin, eight pregnant, and seven lactating. Data common to all reproductive states were subjected to AOV (Proc GLM, v. 8.2, SAS Inst., Inc., Cary, NC) arranged as a 2×3 factorial, with Se chemical form and reproductive state as the main effects, respectively, with a term for the Se chemical form × reproductive state interaction included. When the Se chemical form × reproductive state interaction was significant ($P < 0.05$), preplanned comparisons (least significant difference; $P < 0.05$) of biological significance were conducted. Specifically, Se chemical form differences were determined (selenomethionine vs. selenocystine) within each reproductive state, and reproductive state differences were determined (virgin vs. pregnant vs. lactating) within each Se chemical form treatment. When Se chemical form and/or reproductive state effects, but not the interaction, were significant, means were separated ($P < 0.06$; LSD) within each factor. Data specific to the pregnant and lactating groups were analyzed ($P < 0.05$) within group as a completely randomized design with the two Se chemical forms as the treatments.

Results

The Se chemical form and reproductive state interaction was significant for brain Se concentration ($P = 0.05$; Table 1), but not for heart, kidney, liver, lung, muscle, plasma, spleen, and uterus Se concentrations; heart and liver total Se content; brain, kidney, and liver thioredoxin reductase activities; and brain, erythrocyte, liver, and kidney glutathione peroxidase activities ($P =$ 0.13 to 0.98). Lactating rats consuming selenomethionine had the greatest Se concentration in the brain, with pregnant rats being intermediate, and virgin rats

Table 1. Brain Se concentration of virgin, pregnant, and lactating rats fed 2.0 μg of Se/g of diet (as-fed basis) as either L-selenomethionine or L-selenocystine for 18 d

	Reproductive state				
Response variables[a]	Virgin	Pregnant	Lactating	SE	*P*-value[b]
Brain Se concentration, ng/g[c]					
L-Selenocystine	179.0	205.0	249.3	18.3	0.008
L-Selenomethionine	402.9	460.2	558.0	17.0	0.02
SE	15.9	15.9	17.0	—	—
P-value[d]	<0.001	<0.001	<0.001	—	—

[a]Data are the mean of seven virgin, eight pregnant, and seven lactating rats individually fed selenocystine, and seven virgin, seven pregnant, and seven lactating rats individually fed selenomethionine.
[b]Virgin vs. pregnant vs. lactating (pairwise LSD tests). For L-selenocystine treatment, virgin = pregnant; pregnant = lactating; and virgin < lactating. For L-selenomethionine treatment, virgin < pregnant < lactating.
[c]Fresh-tissue basis.
[d]L-Selenocystine vs. L-selenomethionine (LSD test).

Table 2. Tissue and organ Se load and selenoprotein activities of female rats fed 2.0 μg of Se/g of diet (as-fed basis) as either L-selenocystine or L-selenomethionine for 18 d

	Se chemical form			
Response variables[a]	L-Selenocystine	L-Selenomethionine	SE	*P*-value[b]
Se concentration[c]				
Heart, ng/g	587.6	908.2	35.8	<0.001
Kidney, ng/g	3,294.6	3,390.1	170.7	0.25
Liver, ng/g	2,783.7	3,396.6	107.2	<0.001
Lung, ng/g	829.5	1,125.5	23.9	<0.001
Muscle, ng/g	151.8	465.3	10.5	<0.001
Plasma, ng/mL	606.4	736.3	16.1	<0.001
Spleen, ng/g	1,213.5	1,622.5	57.7	<0.001
Uterus, ng/g	461.7	776.2	18.6	<0.001
Total Se[d]				
Heart, μg	0.627	1.001	0.046	<0.001
Liver, μg	45.25	54.44	2.08	0.003
Thioredoxin reductase[e]				
Brain	15.56	14.02	1.13	0.34
Kidney	22.69	21.68	0.90	0.42
Liver	15.19	15.62	1.09	0.77
Glutathione peroxidase[e]				
Brain	296.8	308.1	11.2	0.13
Erythrocyte	810.1	787.4	38.3	0.85
Kidney	2,508.7	2,461.9	74.6	0.69
Liver	2,291.9	2,594.1	139.3	0.71
Weights, g				
BW	297.1	305.9	5.5	0.25
Heart	1.044	1.085	0.025	0.25
Liver	16.33	16.18	0.54	0.84

[a]Data are the mean of 22 rats individually fed selenocystine, and 21 rats individually fed selenomethionine.
[b]Observed significance level for the comparison of Se forms.
[c]Fresh-tissue basis.
[d]Total Se = tissue weight × Se concentration.
[e]mUnits of activity·mg protein^{-1}·min^{-1}.

having the least ($P = 0.02$). When selenocystine was fed, brain Se concentration was greatest ($P = 0.008$) in lactating rats, and not different between pregnant and virgin rats ($P = 0.34$).

The effects of Se chemical form on Se load and selenoprotein activities are presented in Table 2. The Se concentration in the heart, liver, lung, muscle, plasma, spleen, and uterus were greatest ($P < 0.001$) in rats fed selenomethionine. The total amount (μg; Se concentration × whole tissue weight) of Se contained within the heart and liver was greatest ($P < 0.003$) for rats consuming selenomethionine. Selenium chemical form did not affect ($P = 0.13$ to 0.85) brain, kidney, and liver thioredoxin reductase activities; brain, erythrocyte, kidney, and liver glutathione peroxidase activities; and kidney Se concentration. Liver and heart weights and BW were similar ($P = 0.25$ to 0.84) between Se chemical form treatments.

Presented in Table 3 are the effects of reproductive state on Se load and selenoprotein activities. Lactating rats exhibited the greatest ($P < 0.006$) Se concentration in the heart, lung, muscle, plasma, and spleen. Heart, muscle, and spleen Se concentrations did not differ ($P = 0.13$ to 0.47) between pregnant and virgin rats. Pregnant rats had lower ($P = 0.006$) plasma, but greater ($P = 0.06$) lung Se concentration than virgin rats. The total amount of Se contained within the heart and liver tissues was greatest for lactating rats ($P < 0.003$). Total Se in the liver was greater ($P < 0.003$) for pregnant rats than virgin rats. Thioredoxin reductase activity was greatest ($P < 0.004$) in the brain of pregnant rats and least in lactating and virgin rats, greater ($P < 0.03$) in the kidneys of lactating and pregnant than virgin rats, and greatest in the liver ($P < 0.004$) of lactating rats compared with pregnant and virgin rats. Kidney, brain, liver, and erythrocyte glutathione peroxidase activities, and kidney Se concentration did not differ ($P = 0.12$ to $P = 0.85$) among reproductive states. Total BW was least ($P < 0.01$) in virgin rats, but similar ($P = 0.41$) between pregnant and lactating rats (data not shown).

In pregnant rats (Table 4), uterus (with contents), empty uterus, cumulative placenta, and cumulative and individual fetus weights did not differ ($P = 0.26$ to 0.83) between dietary treatments; however, uterus, placental, and fetal Se concentrations and total Se contents were greater ($P < 0.003$) in rats consuming selenomethionine. There were numerically fewer fetuses ($P = 0.10$) in rats consuming selenomethionine. In the lactating group (Table 5), Se chemical form had no affect ($P = 0.28$ to 0.98) on birth litter count and cumulative weight, weaned litter count and cumulative weight, in-

Table 3. Tissue and organ Se load and selenoprotein activities of virgin, pregnant, and lactating rats fed 2.0 μg of Se/g of diet (as-fed basis) for 18 d

	Reproductive state				
Response variables[a]	Virgin	Pregnant	Lactating	SE	*P*-value[b]
Se concentration[c]					
Heart, ng/g	566.9[v]	649.3[v]	1,027.5[w]	44.0	<0.003
Lung, ng/g	792.8[v]	867.6[w]	1,272.0[x]	29.4	<0.06
Muscle, ng/g	263.3[v]	289.7[v]	372.7[w]	12.9	<0.001
Plasma, ng/mL	689.9[v]	553.5[w]	770.6[x]	19.4	<0.006
Spleen, ng/g	1,212.0[v]	1,318.1[v]	1,723.9[w]	70.9	<0.001
Kidney, ng/g	3,198.5	3,421.7	3,406.8	209.7	0.25
Total Se content					
Heart, μg	0.570[v]	0.669[v]	1.211[w]	0.057	<0.001
Liver, μg	36.56[v]	47.77[w]	65.21[x]	2.55	<0.003
Thioredoxin reductase[d]					
Brain	12.77[v]	18.83[w]	12.76[v]	1.42	<0.004
Kidney	19.12[v]	22.39[w]	25.03[w]	1.15	<0.03
Liver	14.11[v]	12.22[v]	19.89[w]	1.33	<0.004
Glutathione peroxidase[d]					
Brain	299.8	279.4	328.3	14.1	0.12
Erythrocyte	768.0	782.5	845.6	47.3	0.85
Kidney	2,456.4	2,436.4	2,563.1	91.9	0.69
Liver	2,515.3	2,401.1	2,412.6	171.2	0.71

[a]Data are the mean of 14 virgin, 15 pregnant, and 14 lactating rats individually treated with supranutritional Se.
[b]When the *F*-statistic was significant ($P < 0.05$), means were separated using the LSD pairwise test. *P*-values greater than 0.05 are the significance level of the *F*-statistic.
[c]Fresh-tissue basis.
[d]mUnits of activity·mg protein^{-1}·min^{-1}.
[v,w,x]Different superscripts within row indicate a difference, with significance at the *P*-value indicated.

dividual pup weight, or pup liver glutathione peroxidase and thioredoxin reductase activities.

Discussion

Regardless of reproductive state, our data showed that, when provided short term to female rats reared on a low-Se diet, 2.0 μg of Se/g of diet in the form of selenocystine resulted in less Se load than selenomethionine. Selenium concentrations were 67% less in muscle, and ranged from 18 to 41% less in liver, spleen, lung, heart, and uterus when selenocystine was consumed compared with selenomethionine; however, kidney Se concentration did not differ between treatments. The Se distribution patterns we observed for selenocystine were comparable with those previously reported for sodium selenite. For example, 65% less Se was deposited in the muscle tissue of male rats injected (intraperitoneal) with [^{75}Se]selenite compared with [^{75}Se]selenomethionine (Beilstein and Whanger, 1986). Furthermore, Smith and Picciano (1987) reported 16, 26, 29, and 38% less Se in the plasma, kidney, liver, and heart, respectively, of lactating rats fed sodium selenite vs. selenomethionine (0.5 μg of Se/g of diet fed for 18 d).

Supranutritional selenocystine did not induce greater selenoprotein activities than selenomethionine in female rats. Dietary or endogenous seleno-cystine/-cysteine is rapidly metabolized to alanine, and as with dietary sodium-selenite and -selenate, the Se is liberated as selenide (Esaki et al., 1982; Hasegawa et al., 1996; Nakamuro et al., 2000). Therefore, in the short term, supranutritional selenocystine should result in a more rapid contribution of Se to the selenide pool. Selenide is required for selenocysteine incorporation into mammalian selenoproteins; selenocysteyl-tRNA is formed from a unique seryl-tRNA and selenide as a cotranslational event (Beilstein and Whanger, 1986; Sunde, 1990; Stadtman, 1996). Berggren et al. (1999) reported that the specific activity of thioredoxin reductase in male rats increased when 1.0 μg of Se/g of diet was fed as sodium selenite, a rapid contributor to the selenide pool. This response also seemed greater when sodium selenite, as opposed to selenomethionine, was the Se chemical form used in vitro (HT-29 human colon cancer cells; Berggren et al., 1997). Because of the interchangeability between selenomethionine and methionine during translation (Waschulewski and Sunde, 1988; Butler et al., 1989), Se may be temporally sequestered in the general protein pool away from selenide generation. Theoretically, this Se sequestration can only exist until the substitution of methionine with selenomethionine reaches equilibrium (i.e., rate of selenomethionine substitution equals selenomethionine catabolism).

Although we provided supranutritional Se for 17 to 18 d, the lack of difference in enzyme activity between the two Se treatments indicates that equilibrium for selenomethionine metabolism was established, and Se from selenomethionine was readily available for selenocysteine synthesis. Interestingly, when increased thio-

Table 4. Physical characteristics and Se content of the uterus, placentas, and fetuses from pregnant rats fed 2.0 μg of Se/g of diet (as-fed basis) as either L-selenomethionine or L-selenocystine for 18 d

	Se chemical form			
Response variables[a]	L-Selenocystine	L-Selenomethionine	SE	*P*-value[b]
Uterus				
Weight, with contents, g[c]	29.52	26.95	1.52	0.26
Weight, empty, g	4.01	3.77	0.15	0.27
Se, ng/g[d]	471.83	791.5	27.0	<0.001
Total Se, μg[e]	1.87	3.05	0.13	<0.001
Placenta				
Cumulative weight, g	5.37	5.02	0.27	0.36
Se, ng/g[d]	556.9	821.5	31.7	<0.001
Total Se, μg[e]	3.00	4.08	0.21	0.003
Fetus				
Count	13.86	12.43	0.56	0.10
Cumulative weight, g	10.72	10.51	0.68	0.83
Individual weight, g	0.798	0.844	0.039	0.42
Se, ng/g[d]	182.0	453.0	9.9	<0.001
Total Se, μg[e]	1.954	4.798	0.286	<0.001

[a]Data are the mean of eight rats individually fed selenocystine, and seven rats individually fed selenomethionine.
[b]Observed significance level for the comparison of Se forms.
[c]Contents = placentas, fetuses, and fluids.
[d]Fresh-tissue basis.
[e]Total Se = tissue weight × Se concentration.

redoxin reductase activity has been reported, no concomitant rises in mRNA occurred (Berggren et al., 1997; Zhang et al., 2003). As such, previously reported enhancement of thioredoxin reductase activity may be due to sustained and/or increased availability of Se for selenocysteine synthesis during an active translation process (Zhang et al., 2003). Perhaps at 2.0 μg of Se/g of diet fed short term, as opposed to 1.0 μg of Se (Berggren et al., 1997), sufficient dietary selenomethionine is available to sustain or enhance selenocysteine synthesis similar to dietary selenocystine. The lack of Se treatment difference that we observed in Se content of the kidneys indicates that much of the dietary selenomethionine was being readily catabolized.

Independent of the chemical form of Se, reproductive state influenced Se load in the pregnant and lactating rats. Lower plasma Se has been reported in pregnant rats nearing parturition. As parturition approached, plasma Se decreased in rats (Smith and Picciano, 1986), humans (Butler and Whanger, 1992), and cynomolgus macaques (Hawkes et al., 1994). Interestingly, Smith and Picciano (1986) reported that glutathione peroxi-

Table 5. Litter characteristics, liver weight, and selenoprotein activities of pups nursing lactating rats fed 2.0 μg of Se/g of diet (as-fed basis) as either L-selenomethionine or L-selenocystine for 18 d

	Se chemical form			
Response variables[a]	L-Selenocystine	L-Selenomethionine	SE	*P*-value[b]
Litter at birth				
Count	12.86	11.86	0.86	0.43
Cumulative weight, g	79.90	78.12	6.72	0.80
Litter at weaning				
Count	10.86	10.43	0.97	0.79
Cumulative weight, g	291.8	316.8	18.3	0.35
Individual pup weight, g	28.37	31.01	1.72	0.28
Pup liver				
Weight, g	0.914	1.130	0.064	0.03
Glutathione peroxidase[c]	2,754.0	2,802.7	312.1	0.91
Thioredoxin reductase[c]	9.60	9.55	1.11	0.98

[a]Data are the mean of seven rats individually fed selenocystine, and seven rats individually fed selenomethionine.
[b]Observed significance level for the comparison of Se forms.
[c]mUnits of activity·mg protein^{-1}·min^{-1}.

dase activities decreased during pregnancy compared with nonpregnant rats. The lack of enzyme activity decline in the current study may be due to the 10 times greater Se concentration in the diets that were fed in the present experiment. Compared with the virgin and pregnant rats, lactating rats had greatest concentrations of Se found in the plasma, heart, muscle, spleen, and lung. Enhanced N sparing and increased protein synthesis and/or turnover during lactation (Barber et al., 1990) would spare selenomethionine as a methionine-like compound.

Unique to this study was the influence of reproductive state on brain Se and the interaction with Se chemical form. For each advanced reproductive state, brain Se concentration increased (virgin < pregnant < lactating) in the selenomethionine group; however, in the selenocystine group, brain Se concentration was only greater in the lactating rats compared with virgin rats. When selenomethionine was fed supranutritionally and in increasing concentrations (0.2, 1.0, 2.0, and 4.0 μg of Se/g of diet), Whanger and Butler (1988) observed that brain Se concentration in male rats increased according to Se dose; however, when sodium selenite was fed at identical Se concentrations, brain Se did not change. According to the study of Whanger and Butler (1988), and considering the similarity to selenite metabolism, increased intake of selenocystine should not influence brain Se concentrations. As such, the reproductive state of lactation seems to increase brain Se independent from chemical form of dietary Se.

It is unclear as to why reproductive state, as opposed to Se chemical form, influenced thioredoxin reductase activities. Thioredoxin reductase is essential for many redox/antioxidant systems and functions necessary for normal gestation and lactation (Ejima et al., 1999; Arner and Holmgren, 2000; Nordberg and Arner, 2001). Many of these processes, such as intracellular signaling, nutrient metabolism, membrane transport, protein synthesis, and tissue vascular growth, are redox regulated and/or generate a greater oxidative status. A greater oxidant status could trigger an increase in thioredoxin reductase protein. Recently, Hintze et al. (2003) established that the in vitro transcription of the thioredoxin reductase gene is mediated through electrophilic compounds and antioxidant response elements, not Se. However, similar to Zhang et al. (2003), Hintze et al. (2003) found that Se acts synergistically with transcription inducing compounds (i.e., sulforaphane) in vitro to increase thioredoxin reductase activity. Supranutritional Se may act synergistically with the physiological processes of pregnancy and lactation to enhance thioredoxin reductase activity. As previously discussed, the brain Se concentration was greatest in lactating rats, but brain thioredoxin reductase was greatest in pregnant rats. This difference suggests that reproductive state, not Se availability or chemical form, regulates thioredoxin reductase activity in the supranutritional fed rat.

Consistent with maternal Se load, the Se concentration of the placentas and fetuses was greater when selenomethionine was the dietary source of Se, and although not significant ($P = 0.10$), there were numerically fewer fetuses (Table 4). Likewise, the litter count at birth for the lactating rats consuming selenomethionine was numerically less ($P = 0.43$; Table 5). Whether this was a random event is unclear, and caution should be taken when interpreting data based on numerical differences. Nevertheless, in cases involving supranutritional feeding of an element, such occurrences should be considered. As mentioned earlier, the chemical form of Se in commonly fed high-Se grains has been suggested to exist mainly as selenomethionine (Wu et al., 1997; Whanger, 2002). The intentional or inadvertent inclusion of high-Se grains in diets would expose breeding or gestating animals to a substantial amount of selenomethionine. This potential influence of supranutritional selenomethionine in gestating females warrants further investigation.

In conclusion, the short-term feeding of 2.0 μg of Se/g of feed (as-fed basis) in the chemical form of selenomethionine or selenocystine resulted in two greatly different Se distribution patterns and loads in female rats. However, no significant negative effects, for either form, were observed in the female rats or their fetuses or pups. The distribution of Se from supranutritional selenocystine in lactating, pregnant, and virgin rats is similar to the Se from inorganic salts. Furthermore, supranutritional Se form selenomethionine seems readily available for selenoprotein synthesis. Reproductive state, not Se chemical form, seemed to have regulated thioredoxin reductase activity. Regardless of reproductive state, the use of high selenomethionine-containing feeds to provide supranutritional Se short term should result in greater Se load than selenocystine.

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